Periodontal Wound Healing/Regeneration Following Application of Recombinant Human Growth/Differentiation Factor-5 in a ß-Tricalcium Phosphate or an Absorbable Collagen Sponge Carrier into One-Wall Intrabony Defects in Dogs

Young-Taek Kim

The Graduate School

Yonsei University

Department of Dental Science

Periodontal Wound Healing/Regeneration Following Application of Recombinant Human Growth/Differentiation Factor-5 in a ß-Tricalcium Phosphate or an Absorbable Collagen Sponge Carrier into One-Wall Intrabony Defects in Dogs

The Master's Thesis
submitted to the Department of Dental Science
and the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Dental Science

Young-Taek Kim

June 2008

This certifies that the Master's Thesis of

Young-Taek Kim is approved.

_____

Thesis Supervisor:

_____

Thesis Committee Member:

_____

Thesis Committee Member:

The Graduate School

Yonsei University

June 2008

감사의 글

이 논문이 완성되기까지 부족한 저를 항상 격려해주시고 사랑과 관심으로 이끌어 주신 김종관 교수님께 깊은 감사를 드립니다. 그리고 더 나은 논문을 위해 많은 조언과 따뜻한 관심으로 지켜봐 주신 채중규 교수님, 조규성 교수님, 최성호 교수님, 김창성 교수님께 진심으로 감사드립니다.

같이 실험하며 함께 고생한 이중석 선생님, 김태균 선생님, 그리고 우리 파트에서 같이 계측하고 고민한 박정철 선생님에게 감사드립니다. 항상 실험할 때 도움을 주셨던 은영씨와, 의국생활에서 많은 도움을 주었던 의국원들과 특히, 3년을 동고동락한 사랑하는 동기들, 민수, 진혁, 지은, 유정에게도 고마움을 전합니다.

그 밖에 이 실험과 논문에 도움을 준 모든 분들께 감사를 드립니다.

마지막으로, 항상 곁에서 든든하게 후원해주시고, 언제나 끝없는 사랑으로 감싸주시는 아버지, 어머니, 그리고 미국에 있는 누나, 동생에게도 사랑과 고마움을 전합니다.

2008년 6월
저자 씀

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Periodontal Wound Healing/Regeneration Following Application of Recombinant Human Growth/Differentiation Factor-5 in a ß-Tricalcium Phosphate or an Absorbable Collagen Sponge Carrier into One-Wall Intrabony Defects in Dogs

Requirements for biomaterials considered as carriers for biologic agents for tissue engineering in periodontal sites includes applicability, biocompatibility, structural integrity, adequate release kinetics for the biologic agent and bioresorption profile. The objective of this study was to evaluate periodontal wound healing/ regeneration following application of recombinant human growth/differentiation factor-5 in a synthetic, particulate, highly porous, bioresorbable, osteoconductive ß-tricalcium phosphate scaffold (rhGDF-5/ß-TCP) compared to periodontal wound healing/regeneration following application of rhGDF-5 in an absorbable collagen sponge (rhGDF-5/ACS) matrix in an one-wall intrabony defect model in the Beagle dog.

Unilateral, critical-size (5-mm), one-wall, intrabony periodontal defects were surgically created in the mandibular premolar region in ten Beagle dogs. Unilateral

defects in five animals received rhGDF-5/ß-TCP while the remaining five animals similarly received rhGDF-5/ACS. The animals were euthanized for hisopathologic and histiometric analysis following an 8-week healing interval.

rhGDF-5/ACS and rhGDF-5/ß-TCP groups all showed enhanced bone formation. G-TCP group showed better bone formation in bone height and bone volume than G-ACS groups.

rhGDF-5/ß-TCP showed significant enhanced bone formation. rhGDF-5/ß-TCP seems to releases rhGDF-5 over a long period of time as well as serving as a space maintainer. These results suggest that β-TCP appears as suitable carrier for rhGDF-5 for periodontal inlay indications.

Key words : rhGDF-5, tissue engineering, periodontal regeneration, β-TCP, absorbable collagen sponge, dog

Periodontal Wound Healing/Regeneration Following Application of Recombinant Human Growth/Differentiation Factor-5 in a ß-Tricalcium Phosphate or an Absorbable Collagen Sponge Carrier into One-Wall Intrabony Defects in Dogs

Young-Taek Kim, D.D.S.

Department of Dental Science
Graduate School, Yonsei University
(directed by prof. Chong-Kwan Kim, D.D.S., M.S.D., PhD.)

I. INTRODUCTION

For tissue engineering, we should consider three determinants, cells, scaffolds, and signaling molecules (Babensee et al. 2000; Bartold et al. 2006). Blood supplies could be an additional factor for tissue engineering. Since cells and blood supplies are not controllable factors, scaffolds and signaling molecules would be the true determinants for the investigators and clinicians.

The bone morphogenetic protein (BMP) acting as a signaling molecule, is now regarded as a good additive for periodontal regeneration or bone regeneration (Wang

et al. 1990; Sampath et al. 1992; Ripamonti et al. 1994; Giannobile et al. 2003; Taba et al. 2005). Studies have shown that BMP-2, -4, -5, -6 and -7 have osteoinductive properties (Yamaguchi et al. 1991; Chang et al. 1994; King et al. 1997). However, these BMPs showed complications like root resorption or ankylosis (Saito et al. 2003; Wikesjo et al. 2004), while GDF-5, -6 and -7 (BMP-14, -13, -12) are known as cartilage and bone inducing proteins without root resoprtion and ankylosis. Recombinant human growth/differentiation factor-5 (rhGDF-5), a member of the transforming growth factor-β superfamily was reported as a suitable factor for enhancing healing in bone defect and ectopic bone formation (Spiro et al. 2000; Yoshimoto et al. 2006).

The absorbable collagen is regarded as a gold standard of a BMP carrier (McPherson 1992; Uludag et al. 2001; Kleinman et al. 2003). However it has a significant limitation that it easily collapses and is absorbed too early to release enough signaling molecules (Bartold et al. 2000; King et al. 2002; Bartold et al. 2006). New forms of carriers are required to overcome these two aspects. Currently many resorbable scaffolds chemically synthesized are being introduced. Polyglycolic acid (PLA), polylactic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), Hydroxyapatite, and β-Tricalcium phosphate (β-TCP) are those (Giannobile 1996; Babensee et al. 2000; Taba et al. 2005; Bartold et al. 2006). Growth factor requires appropriate

scaffolds to apply to defect sites.

β-TCP is a bioresorbable and biocompatible material (Urist et al. 1984; Urist et al. 1987; Jung et al. 2006; Poehling et al. 2006), and it is expected to maintain the space long enough for the bone regeneration. Growth and differentiation factor-5 is also named cartilage-derived morphogenic protein-1 (CDMP-1) and its properties of chondrogenesis, osteogenesis and angiogenesis are being reported (Yamashita et al. 1997; Spiro et al. 2000; Kuniyasu et al. 2003; Poehling et al. 2006; Yoshimoto et al. 2006; Zeng et al. 2006). In the present study, the materials used are Beta-Tricalcium phosphate (β-TCP) coated with recombinant human growth/differentiation facor-5 (rhGDF-5) (Poehling et al. 2006). The aim of this study was to estimate the effect of rhGDF-5 with β-TCP carrier (G-TCP) compared to ACS carrier (G-ACS) in the beagle dog 1-wall defect model.

II. MATERIALS AND METHODS

A. Animals

Ten male Beagle dogs, approximately 15 months old, weight 10-15 kg, bred exclusively for biomedical research purposes, were used. The animals exhibited an intact dentition with a healthy periodontium. Animal selection and management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. The animals had *ad libitum* access to water and a pelleted laboratory diet with the exception of one week immediately postsurgery when they were fed a canned soft dog food diet (Prescription Diet Canine i/d, Hill's Pet Nutrition, Inc., Topeka, Kansas, USA).

B. Materials

G-TCP is composed of β-TCP carrier that is coated with homogeneously with rhGDF-5. The β-TCP particle size ranges between 500 and 1000 μm featuring interconnecting porosity. The rhGDF-5 dose was 500 μg/gram β-TCP.

G-ACS is the absorbable collagen sponge[§], which is soak-laoded with rhGDF-5.

The collagen sponges were natural-born and bio-absorbable. The applied dose is estimated approximately same as G-TCP, 20 μg rhGDF-5 per each defect.

C. Surgical Procedures

All surgical procedures including extraction and experiment were performed under general anesthesia. Induction by intravenous injection of atropin[‖] and intramuscular injection of a combination of xylazine[¶] and ketamin[#] were performed and the general anesthesia was maintained with inhalation anesthesia[**].

After the extraction of first premolar and third premolar, 8 weeks were given for complete socket healing. After the infiltration anesthesia, full thickness mucoperiosteal flap was elevated to make 1-wall defect at the pre-extraction site. The defects surgically created as "box-type" (4 mm width, 5 mm depth), one-wall, intrabony defects were made distal to the first premolar and mesial to the third premolar(Kim, H. Y. et al. 2002; Kim, C. S. et al. 2005). Defects in the test group were filled with G-TCP. In the other five dogs, G-ACS was applied.

Next, the mucoperiosteal flaps were advanced, adapted, and sutured with resorbable suture materials[††].

Post-surgical management included intramuscular administration of antibiotics[‡‡] for 3 days and daily topical dressing of 0.2% chlorhexidine solution[§§] for infection control for 7 days.

The extraction sites were allowed to heal for 2 months. The remaining dentition received oral prophylaxis in conjunction with the extraction procedures. The animals were euthanized 8 weeks following the first surgical procedure and block sections including the surgical sites were removed for the histologic analysis.

D. Clinical and Radiographic Records

Clinical photos and radiographs were taken at the time of the extraction, the surgery and the necropsy. At each time, before and after photos and radiographs were recorded.

E. Histologic & Histometric Analysis

The animals were sacrificed using an overdose of pentobarbital (90 -120 mg/kg; IV). Block sections including defect sites and tooth, surrounding alveolar bone and soft tissues were collected. The block specimens were fixed in 10% buffered formalin for 10 days, decalcified in 5% nitric acid for 7 days, trimmed, dehydrated and

embedded in paraffin. Serial sections, 4µm thick, were cut in a mesial-distal direction at 80µm intervals. The sections were stained using hematoxylin and eosin.

The three most central sections of each defect site were observed using incandescent and polarized light microscopy‖ ‖. Histometric analysis was performed using image analysis software¶¶. The following parameters were recorded (Figure 2).

- Defect height: distance from the apical extension of the root surface notch to the cemento-enamel junction (CEJ);
- Epithelial attachment: distance from the CEJ to the apical extension of an epithelial attachment on the root surface. This parameter included any gingival recession.
- Cementum regeneration: distance from the apical extension of the root surface notch to the coronal extension of newly formed cementum or a cementum-like substance on the root surface.
- Bone regeneration (height): distance from the apical extension of the root surface notch to the coronal extension of newly formed bone along the root surface;
- Bone regeneration (area): new alveolar bone within the standardized template that served as a proxy for the defect site. The template was aligned parallel to the root surface interfacing the apical extension of defect at the root surface notch.
- Root resorption

o Ankylosis

F. Statistical Analysis

The experimental groups were G-TCP groups were compared to the G-ACS groups using student *t*-test ($p < 0.05$) with Statistics software[##].

§ Colla-tape®, Zimmer Dental, Carlsbad, CA, USA
‖ 0.04 mg/kg; Kwangmyung Pharmaceutical Ind. Co. Ltd., Seoul, Korea
¶ Rompun, Bayer Korea Co., Seoul, Korea
# Ketara, Yuhan Co., Seoul, Korea
** Gerolan, Choongwae Pharmaceutical Co., Seoul, Korea
†† Vicryl 5.0 Polyglactin 910, Ethicon, Johnson & Johnson, New Jersey, USA
‡‡ Cefazoline Sodium 20mg/kg; Yuhan Corporation, Seoul, Korea
§§ Hexamedin®, Bukwang Pharmaceutical Co., Seoul, Korea
‖ ‖ Olympus Multi-view microscope BH2, Tokyo, Japan
¶¶ Image-Pro Plus, Media Cybernetic, Silver Springs, MD, USA
Microsoft Office Excel 2003, Microsoft Co., Redmond, WA, USA

III. RESULTS

A. Clinical & Radiographic Observations

No sign of infection or other clinical complications were observed during the eight-week healing interval. Figure 1 shows the clinical observations at surgery and necropsy for each group. Figure 3 shows representative radiographs of the each group at the time of surgery and necropsy suggesting greater bone formation at defect sites implanted with G-TCP compared to G-ACS.

B. Histologic & Histometric Analysis

Results from the histometric analysis are shown in Table 1. The defect height averaged (± SD) 5.01 ± 0.51 mm and 4.58 ± 0.54 mm respectively for the defects receiving the G-ACS, and G-TCP with no significant differences between the treatments ($p > 0.05$). The junctional epithelium was 1.55 ± 1.18 mm and 0.65 ± 0.33 mm for the defects receiving the G-ACS and G-TCP respectively without significant differences. Amount of the cementum regeneration averaged 3.03 ± 1.18 and 3.83 ± 0.73mm respectively without significant differences.

Dense fibers showing periodontal regeneration were embedded into the newly

formed bone and new cellular cementum obliquely or perpendicularly (Figure 5).

Mean values of bone regeneration show 2.22 ± 0.82 mm and 3.26 ± 0.30 mm for G-ACS and G-TCP respectively with significant differences between two groups ($p < 0.05$). Significant differences between 2 groups were shown in bone regeneration volumes ($p < 0.05$). G-TCP showed greater regenerated bone height and greater regenerated bone volume than G-ACS ($p < 0.05$). The woven nature of the new bone was shown and it appeared hypercellularity and higher density in both G-TCP and G-ACS groups (Figure 6).

IV. DISCUSSION

Periodontal defects can be restored in many ways. Different methods such as resective therapies, bone grafting, and guided tissue/bone regeneration were tried (Giannobile 1996; Taba et al. 2005; Bartold et al. 2006). Recently, a combination of several techniques was introduced as the tissue engineering technique improves (Bartold et al. 2000; Bartold et al. 2006). Bone morphogenetic proteins or growth factors have been studied in this aspect. RhGDF-5 is one of the bone morphogenetic protein family of the TGF-β superfamily and they were reported as having abilities of chondrogenesis, osteogenesis and angiogenesis (Francis-West et al. 1999). RhGDF-5 displays strong osteoinductive ability with limited risk of excessive bone formation (Kuniyasu et al. 2003). In the present study, G-ACS group and G-TCP group showed 44.31% and 71.18% of new bone formation respectively and G-TCP had significantly more bone formation than G-ACS. This result supports that the rhGDF-5 has the ability of osteogenesis, assuring previous literatures (Yamashita et al. 1997; Spiro et al. 2000; Kuniyasu et al. 2003; Poehling et al. 2006; Yoshimoto et al. 2006; Zeng et al. 2006). Ankylosis or root resorption was not observed in the present study.

As well as bone formation, periodontal regeneration is one of the goals for tissue

engineering. For measuring the periodontal regeneration, cementum regeneration was measured and periodontal ligament was observed. In the cementum regeneration, G-ACS and G-TCP group showed 60.47% and 83.62% of new cementum formation with no significant difference between two groups. Compared to 30 to 35% of cementum regenetation in 1-wall defect without treatment (Kim, H. Y. et al. 2002; Kim, C. S. et al. 2004; Kim, C. S. et al. 2005), it is assumed that G-ACS and G-TCP both have the enhanced cementum regeneration, which implicates the periodontal regeneration. In histologic observation, dense and well organized form of periodontal ligaments was shown. RhGDF-5 seems to be very effective in periodontal regeneration with these two carriers.

BMPs and proteins are applied to periodontal or bony defects with or without delivery devices. Not only fillers but also many other materials are developed as delivery devices, so called, carriers. In this study, the carrier was brought into focus. The delivery devices should have several characteristics. It should be: (1) porous, to allow cell infiltration; (2) biocompatible, to minimize inflammatory reactions; and (3) biodegradable, so as not to interfere with the long-term properties of the repaired tissue (Seeherman 2001). The absorbable collagen sponge has been a gold standard as a carrier (McPherson 1992). It has been utilized as a biomaterial, which allows fibroblast invasion, neovascularization and epidermal regeneration. And its releasing

time could be also somewhat controlled. But it has limitations on several aspects. The absorbable collagen sponge is easily collapsed. That is, the space for periodontal or bone regeneration is not maintained for an appropriate time. The other possibility is the short releasing time. Although some articles say the releasing time could be controlled, the time is short and it cannot be said that it release the BMPs or growth factors proportionally (McPherson 1992; Uludag et al. 2001; Kleinman et al. 2003). So many other biomaterials were developed and studied. β-TCP is the one of them. It is slowly absorbed and its framework makes the space maintained for enough time. Most of all, many methods to make proteins and growth factors released from β-TCP slowly were developed (Fournier et al. 1996; Whang et al. 1998; Liu et al. 2006; Park et al. 2006). RhGDF-5 is released in a controlled fashion over a period of 7 days. The sustained release characteristics of rhGDF-5 from its β-TCP carrier may contribute to the effectiveness of G-TCP (Poehling et al. 2006). In the present study, by comparing the absorbable collagen sponge and the β-TCP as a carrier, it showed significant differences in bone regeneration and bone area. Mean values of bone regeneration showed 2.22 ± 0.82, 3.26 ± 0.30 mm G-ACS, G-TCP respectively with significant differences between two groups ($p<0.05$). Significant differences in bone regeneration volumes ($p<0.05$) between 2 groups were also shown. Percentages of bone formation in G-TCP were 71.18%, which was superior to that of G-ACS, 44.31%. While in both two groups, hypercellularity and high density of new bone were observed in the

histologic observation, the reason for this result seems to be due to two properties mentioned.

RhGDF-5/β-TCP seems to release rhGDF-5 over a long period of time as well as serving as a space maintainer. A framework for bone ingrowth aids in preventing the collapse of the soft tissues and promotes stabilization of the blood clot. These results suggest that β-TCP appears as a suitable carrier of rhGDF-5 for periodontal inlay indications.

V. CONCLUSION

In conclusion, G-TCP release the rhGDF-5 over a long period of time as well as it serves as the space maintaining. But, in this study it is not certain of proportional releasing for a long period of time. So, further studies may be needed. Nevertheless, these results showed that β-TCP can be a good candidate of the rhGDF-5 carrier.

REFERENCES

Babensee, J. E., McIntire, L. V. and Mikos, A. G. Growth factor delivery for tissue engineering. *Pharm Res* 17(5): 497-504, 2000.

Bartold, P. M., McCulloch, C. A., Narayanan, A. S. and Pitaru, S. Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. *Periodontol 2000* 24: 253-269, 2000.

Bartold, P. M., Xiao, Y., Lyngstaadas, S. P., Paine, M. L. and Snead, M. L. Principles and applications of cell delivery systems for periodontal regeneration. *Periodontol 2000* 41: 123-135, 2006.

Chang, S. C., Hoang, B., Thomas, J. T., Vukicevic, S., Luyten, F. P., Ryba, N. J., Kozak, C. A., Reddi, A. H. and Moos, M., Jr. Cartilage-derived morphogenetic proteins. New members of the transforming growth factor-beta superfamily predominantly expressed in long bones during human embryonic development. *J Biol Chem* 269(45): 28227-28234, 1994.

Fournier, N. and Doillon, C. J. Biological molecule-impregnated polyester: an in vivo angiogenesis study. *Biomaterials* 17(17): 1659-1665, 1996.

Francis-West, P. H., Abdelfattah, A., Chen, P., Allen, C., Parish, J., Ladher, R., Allen, S., MacPherson, S., Luyten, F. P. and Archer, C. W. Mechanisms of GDF-5 action during skeletal development. *Development* 126(6): 1305-1315, 1999.

Giannobile, W. V. Periodontal tissue engineering by growth factors. *Bone* 19(1 Suppl): 23S-37S, 1996.

Giannobile, W. V. and Somerman, M. J. Growth and amelogenin-like factors in periodontal wound healing. A systematic review. *Ann Periodontol* 8(1): 193-204, 2003.

Jung, U. W., Choi, S. Y., Pang, E. K., Kim, C. S., Choi, S. H. and Cho, K. S. The effect of varying the particle size of beta tricalcium phosphate carrier of recombinant human bone morphogenetic protein-4 on bone formation in rat calvarial defects. *J Periodontol* 77(5): 765-772, 2006.

Kim, C. S., Choi, S. H., Chai, J. K., Cho, K. S., Moon, I. S., Wikesjo, U. M. and Kim,

C. K. Periodontal repair in surgically created intrabony defects in dogs: influence of the number of bone walls on healing response. *J Periodontol* 75(2): 229-235, 2004.

Kim, C. S., Choi, S. H., Cho, K. S., Chai, J. K., Wikesjo, U. M. and Kim, C. K. Periodontal healing in one-wall intra-bony defects in dogs following implantation of autogenous bone or a coral-derived biomaterial. *J Clin Periodontol* 32(6): 583-589, 2005.

Kim, H. Y., Kim, C. S., Jhon, G. J., Moon, I. S., Choi, S. H., Cho, K. S., Chai, J. K. and Kim, C. K. The effect of safflower seed extract on periodontal healing of 1-wall intrabony defects in beagle dogs. *J Periodontol* 73(12): 1457-1466, 2002.

King, G. N. and Cochran, D. L. Factors that modulate the effects of bone morphogenetic protein-induced periodontal regeneration: a critical review. *J Periodontol* 73(8): 925-936, 2002.

King, G. N., King, N., Cruchley, A. T., Wozney, J. M. and Hughes, F. J. Recombinant human bone morphogenetic protein-2 promotes wound healing in rat periodontal fenestration defects. *J Dent Res* 76(8): 1460-1470, 1997.

Kleinman, H. K., Philp, D. and Hoffman, M. P. Role of the extracellular matrix in morphogenesis. *Curr Opin Biotechnol* 14(5): 526-532, 2003.

Kuniyasu, H., Hirose, Y., Ochi, M., Yajima, A., Sakaguchi, K., Murata, M. and Pohl, J. Bone augmentation using rhGDF-5-collagen composite. *Clin Oral Implants Res* 14(4): 490-499, 2003.

Liu, H. W., Chen, C. H., Tsai, C. L. and Hsiue, G. H. Targeted delivery system for juxtacrine signaling growth factor based on rhBMP-2-mediated carrier-protein conjugation. *Bone* 39(4): 825-836, 2006.

McPherson, J. M. The utility of collagen-based vehicles in delivery of growth factors for hard and soft tissue wound repair. *Clin Mater* 9(3-4): 225-234, 1992.

Park, Y. J., Kim, K. H., Lee, J. Y., Ku, Y., Lee, S. J., Min, B. M. and Chung, C. P. Immobilization of bone morphogenetic protein-2 on a nanofibrous chitosan membrane for enhanced guided bone regeneration. *Biotechnol Appl Biochem* 43(Pt 1): 17-24, 2006.

Poehling, S., Pippig, S. D., Hellerbrand, K., Siedler, M., Schutz, A. and Dony, C.

Superior effect of MD05, beta-tricalcium phosphate coated with recombinant human growth/differentiation factor-5, compared to conventional bone substitutes in the rat calvarial defect model. *J Periodontol* 77(9): 1582-1590, 2006.

Ripamonti, U. and Reddi, A. H. Periodontal regeneration: potential role of bone morphogenetic proteins. *J Periodontal Res* 29(4): 225-235, 1994.

Saito, E., Saito, A. and Kawanami, M. Favorable healing following space creation in rhBMP-2-induced periodontal regeneration of horizontal circumferential defects in dogs with experimental periodontitis. *J Periodontol* 74(12): 1808-1815, 2003.

Sampath, T. K., Maliakal, J. C., Hauschka, P. V., Jones, W. K., Sasak, H., Tucker, R. F., White, K. H., Coughlin, J. E., Tucker, M. M., Pang, R. H. and et al. Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. *J Biol Chem* 267(28): 20352-20362, 1992.

Seeherman, H. The influence of delivery vehicles and their properties on the repair of segmental defects and fractures with osteogenic factors. *J Bone Joint Surg Am* 83-A

Suppl 1(Pt 2): S79-81, 2001.

Spiro, R. C., Liu, L., Heidaran, M. A., Thompson, A. Y., Ng, C. K., Pohl, J. and Poser, J. W. Inductive activity of recombinant human growth and differentiation factor-5. *Biochem Soc Trans* 28(4): 362-368, 2000.

Taba, M., Jr., Jin, Q., Sugai, J. V. and Giannobile, W. V. Current concepts in periodontal bioengineering. *Orthod Craniofac Res* 8(4): 292-302, 2005.

Uludag, H., Gao, T., Porter, T. J., Friess, W. and Wozney, J. M. Delivery systems for BMPs: factors contributing to protein retention at an application site. *J Bone Joint Surg Am* 83-A Suppl 1(Pt 2): S128-135, 2001.

Urist, M. R., Lietze, A. and Dawson, E. Beta-tricalcium phosphate delivery system for bone morphogenetic protein. *Clin Orthop Relat Res* (187): 277-280, 1984.

Urist, M. R., Nilsson, O., Rasmussen, J., Hirota, W., Lovell, T., Schmalzreid, T. and Finerman, G. A. Bone regeneration under the influence of a bone morphogenetic protein (BMP) beta tricalcium phosphate (TCP) composite in skull trephine defects in dogs. *Clin Orthop Relat Res* (214): 295-304, 1987.

Wang, E. A., Rosen, V., D'Alessandro, J. S., Bauduy, M., Cordes, P., Harada, T., Israel, D. I., Hewick, R. M., Kerns, K. M., LaPan, P. and et al. Recombinant human bone morphogenetic protein induces bone formation. *Proc Natl Acad Sci U S A* 87(6): 2220-2224, 1990.

Whang, K., Tsai, D. C., Nam, E. K., Aitken, M., Sprague, S. M., Patel, P. K. and Healy, K. E. Ectopic bone formation via rhBMP-2 delivery from porous bioabsorbable polymer scaffolds. *J Biomed Mater Res* 42(4): 491-499, 1998.

Wikesjo, U. M., Sorensen, R. G., Kinoshita, A., Jian Li, X. and Wozney, J. M. Periodontal repair in dogs: effect of recombinant human bone morphogenetic protein-12 (rhBMP-12) on regeneration of alveolar bone and periodontal attachment. *J Clin Periodontol* 31(8): 662-670, 2004.

Yamaguchi, A., Katagiri, T., Ikeda, T., Wozney, J. M., Rosen, V., Wang, E. A., Kahn, A. J., Suda, T. and Yoshiki, S. Recombinant human bone morphogenetic protein-2 stimulates osteoblastic maturation and inhibits myogenic differentiation in vitro. *J Cell Biol* 113(3): 681-687, 1991.

Yamashita, H., Shimizu, A., Kato, M., Nishitoh, H., Ichijo, H., Hanyu, A., Morita, I.,

Kimura, M., Makishima, F. and Miyazono, K. Growth/differentiation factor-5 induces angiogenesis in vivo. *Exp Cell Res* 235(1): 218-226, 1997.

Yoshimoto, T., Yamamoto, M., Kadomatsu, H., Sakoda, K., Yonamine, Y. and Izumi, Y. Recombinant human growth/differentiation factor-5 (rhGDF-5) induced bone formation in murine calvariae. *J Periodontal Res* 41(2): 140-147, 2006.

Zeng, Q., Li, X., Beck, G., Balian, G. and Shen, F. H. Growth and differentiation factor-5 (GDF-5) stimulates osteogenic differentiation and increases vascular endothelial growth factor (VEGF) levels in fat-derived stromal cells in vitro. *Bone*, 2006.

TABLE

Table 1. Histomorphometric analysis (group means ± SD in mm/mm^2).

	rhGDF-5/ß-TCP	rhGDF-5/ACS	p-value
Defect height	4.58 ± 0.54	5.01 ± 0.51	ns
Epithelial attachment	0.65 ± 0.33	1.55 ± 1.18	ns
Connective tissue	0.10 ± 0.10	0.43 ± 0.85	ns
Cementum regeneration	3.83 ± 0.73	3.03 ± 1.18	ns
Bone regeneration (height)	3.26 ± 0.30	2.22 ± 0.82	< 0.05
Bone regeneration (area)	10.45 ± 2.26	5.48 ± 1.40	< 0.05

FIGURE LEGENDS

Figure 1. Surgically created, critical-size, one-wall, intrabony periodontal defect at the distal aspect of the mandibular 2nd and mesial aspect of the mandibular 4th premolar teeth (left). Application of rhGDF-5/ß-TCP (left center top) or rhGDF-5/ACS (left center bottom). Mucoperiosteal flaps adapted and sutured for primary intention healing (right center). Healing at week 8 (right).

Figure 2. Landmarks/parameters used in the histometric analysis. The green template served as a proxy for the defect site for estimation of the bone regeneration area.

Figure 3. Representative radiographs showing defect sites following implantation of rhGDF-5/β-TCP (top left) and rhGDF-5/ACS (top right) immediately postsurgery and at 8 weeks (bottom left and right, respectively).

Figure 4. Photomicrographs from defect sites receiving rhGDF-5/β-TCP (left) and rhGDF-5/ACS (right) at 8 weeks. Greater bone formation can be observed at implanted with rhGDF-5/β-TCP compared to rhGDF-5/ACS. Aa/Ba show the apical extent of a junctional epithelium, Ab/Bb the coronal extension of newly formed alveolar bone, and Ac/Bc the apical extension (notch) of the defect sites. A few

residual ß-TCP particles surrounded by new bone formation can be observed (A and Ac). (hematoxylin/eosin, original magnification X10 & X40).

Figure 5. Photomicrographs from defect sites receiving rhGDF-5/β-TCP (left) and rhGDF-5/ACS (right) at 8 weeks. Oblique or perpendicular oriented PDL fibers are inserted into newly formed bone and cementum. The PDL appears denser and well-organized at the site receiving rhGDF-5/β-TCP (hematoxylin/eosin, original magnification X200).

Figure 6. Photomicrographs from defect sites receiving rhGDF-5/β-TCP (left) and rhGDF-5/ACS (right) at 8 weeks characterized by dense, hypercellular, woven bone (hematoxylin/eosin, original magnification X100).

Figure 7. Main results from the histometric analysis (* $p < 0.05$).

FIGURES

Figure 1. Clinical photographs

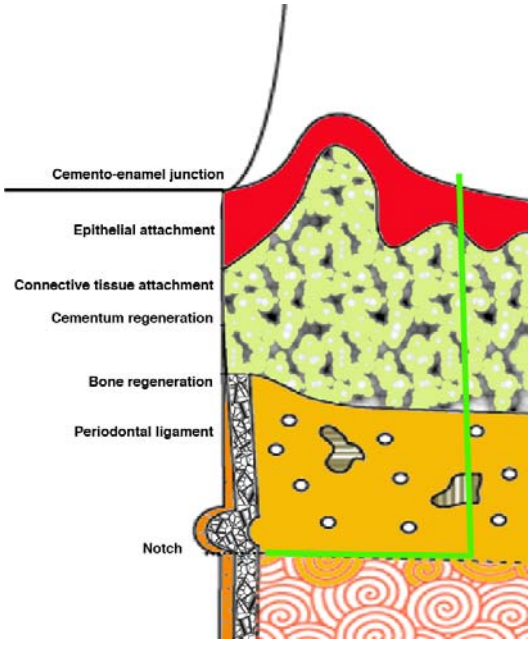

Figure 2. Landmarks/parameters used in the histometric analysis

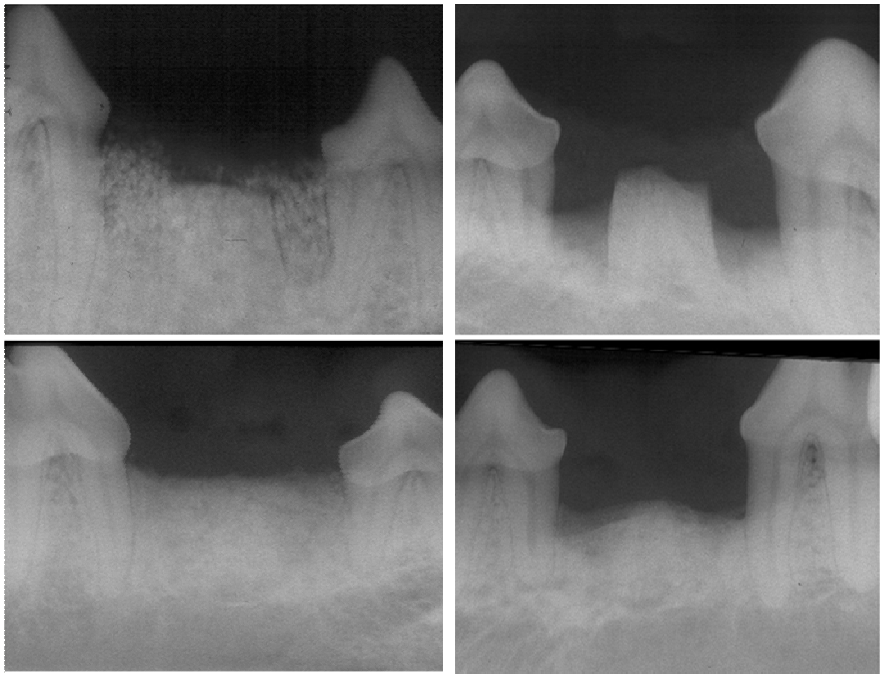

Figure 3. Radiographs of sites

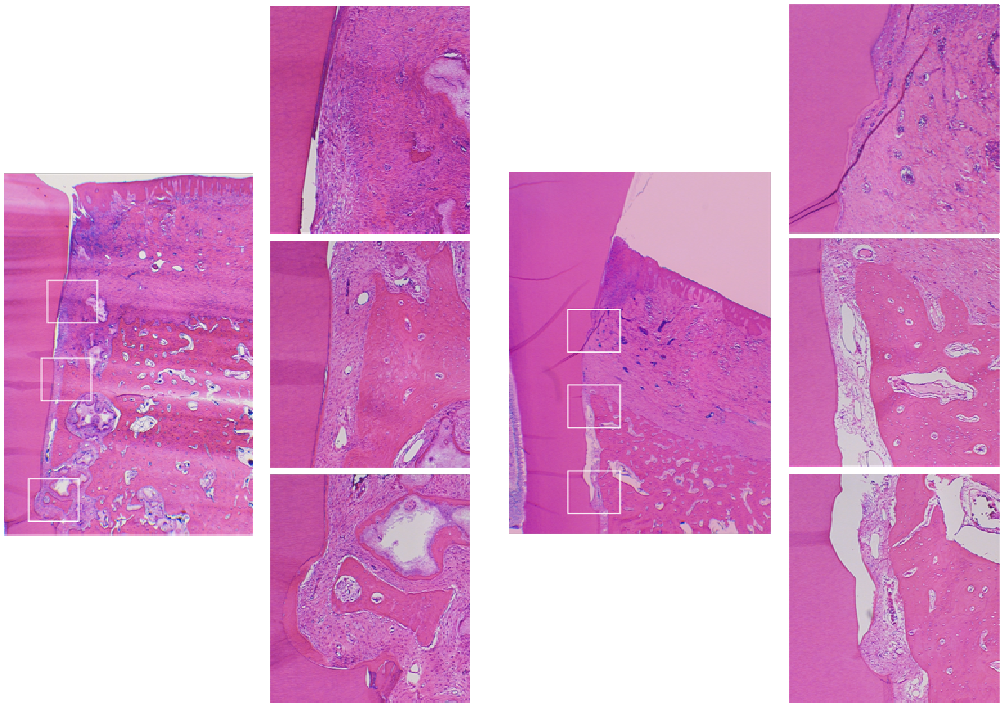

Figure 4. Microphotographs of defect sites(x40, x10)

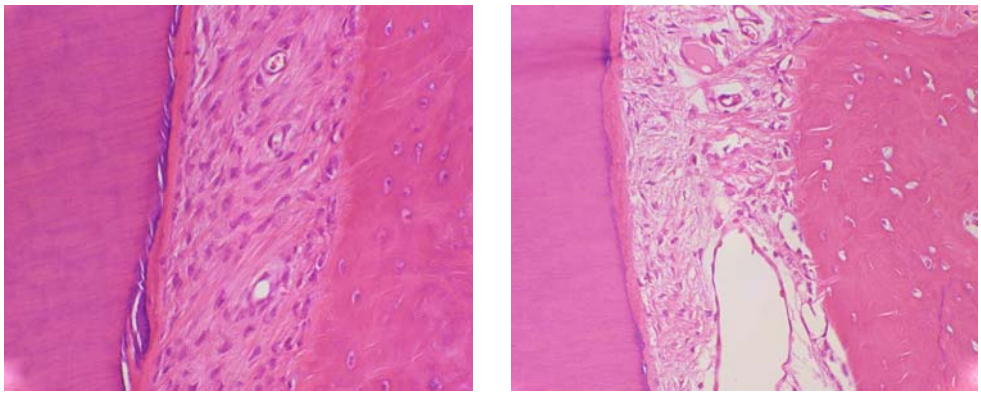

Figure 5. Microphotographs of Periodontal ligaments (x200)

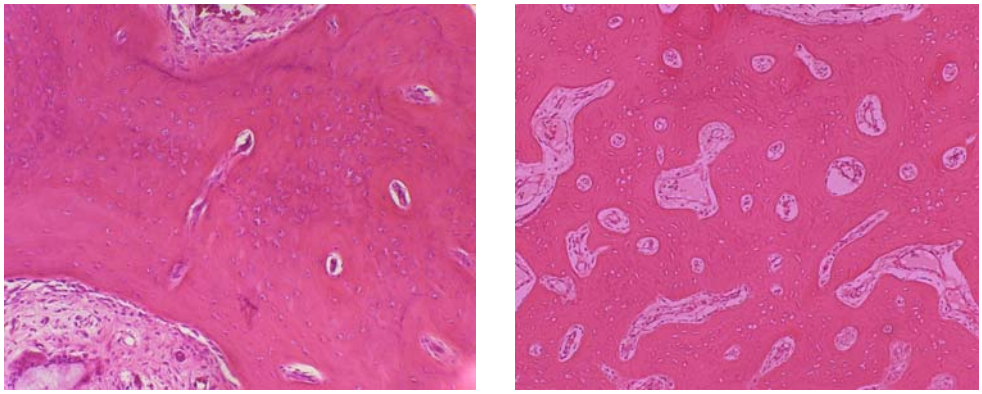

Figure 6. Microphotographs of new bones (x100)

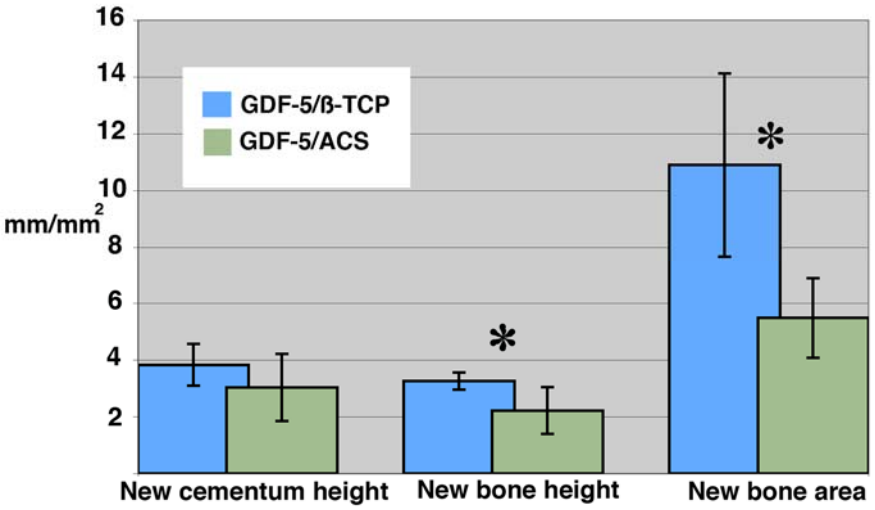

Figure 7. Histometric analysis

국문요약

성견 일벽성 골내낭에서 rh-Growth/Differentiation Factor-5의 운반체로 ß-Tricalcium Phosphate와 Absorbable Collagen Sponge 적용에 따른 치주조직 재생

< 지도 교수 **김 종 관** >

연세대학교 대학원 치의학과

김 영 택

조직공학 측면에서 치주조직 재생을 위한 세가지 요소(cells, scaffolds, signaling molecules) 중 Scaffold로 쓰이는 재료는 조작이 용이해야 하며, 생체적합성이 있어야 하고, 구조적인 안정성을 가져야 한다. 또한, signaling molecule을 운반하는데 있어서 적절한 속도로 분비해야 하며, 그 자신 또한 염증반응없이 흡수되어야 한다. 이 실험의 목적은 recombinant human growth/differentiation factor-5(rhGDF-5)를 적용함에 있어서 골형성유도 단백질을 운반하는 'gold standard'로 여겨지는 absorbable collagen sponge(ACS)와, 합성물질이며 생체적합성이 있으며 구조적인 안정성을 갖는 beta-tricalcium phosphate(ß-

TCP)를 비글견의 일벽성 골내낭에 적용하여 치주조직 치유와 재생의 정도를 비교하고자 하는 것이다.

10마리의 비글견에서 제3소구치를 모두 발치한 뒤, 8주간의 치유기간이 지나고, 제2소구치 원심면과 제4소구치 근심면에 5mm 깊이, 넓이의 일벽성 골내낭을 형성한다. 5마리는 rhGDF-5/ß-TCP를 적용하고, 나머지 5마리는 rhGDF-5/ACS를 적용한다. 8주간의 치유기간이 지난 뒤, 조직학적인 분석과 조직계측적인 분석을 위해서 희생하였다.

조직학적인 분석 결과, rhGDF-5/ß-TCP와 rhGDF-5/ACS, 두군 모두에서 주목할 만한 골 및 백악질의 형성을 보였다. 하지만, 조직계측학적 분석 결과, rhGDF-5/ß-TCP군에서는 골형성 높이와 골형성 넓이 모두에서 rhGDF-5/ACS군에 비해 유의성있는 증가를 보였다.

rhGDF-5/ß-TCP군이 보인 유의성있는 골형성은 ACS에 비하여 ß-TCP가 공간을 유지하는 역할을 할뿐만 아니라, rhGDF-5를 오랜 기간 방출하는 역할을 하기 때문인 것으로 보인다.

이러한 결과는 ß-TCP가 치주 결손부위의 재생에 있어서 ACS를 대체하여, rhGDF-5를 위한 적절한 운반체로써 쓰일 수 있음을 보여준다.

핵심되는 말 : rhGDF-5, 조직공학, 치주재생, ß-TCP, 흡수성 콜라겐